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First record of *Anatololacerta pelasgiana* (Mertens, 1959) in mainland Greece: another new species in Athens

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Abstract

Urban habitats receive an increasing number of species due to anthropogenic activities, mainly transportations. Here, we report a new addition to the herpetofauna of Athens (Greece): a small population of the Pelasgian wall lizard (*Anatololacerta pelasgiana*) was found in a suburb of the Athenian metropolitan area. The species normally occurs in southwestern Anatolia and southeastern Aegean islands and this is the first record in the Greek mainland. Allochthonous species that successfully colonize cities raise new challenges to urban ecology.

Key Words

introduction, lizard, Mediterranean, phylogenetic analysis, urban ecology

The lacertid genus *Anatololacerta* comprises five species according to the latest phylogenetic review of the taxon (Karakasi et al. 2021). Three of them occur both in Turkey and in some southeastern Greek islands: *A. anatolica* (Werner, 1900) in Samos and Ikaria, *A. finikensis* (Eiselt & Schmidtler, 1986) in Psomi islet and *A. pelasgiana* (Mertens, 1959) in Kastelorizo, Rhodes, Symi and surrounding islets (Fig. 1; Karakasi et al. 2021; Uetz et al. 2021). The Pelasgian rock lizard is a small bodied (SVL up to 65 mm) diurnal species that feeds on invertebrates, mainly insects (Valakos et al. 2008). The species prefers rocky areas, cultivated lands with dry-stone walls, and light deciduous forests, while it also frequents small human settlements and ruins where it can be seen climbing on the walls (Lymberakis et al. 2018).

On 27 November 2020, we visited the western outskirts of Athens for a herpetological survey. The broader area, known collectively as Elaionas (*Ελαιώνας*, literally olive grove), the historical part of Athens where olive trees were cultivated since the antiquity is nowadays a degraded district hosting small industries and logistics companies together with abandoned buildings and uncultivated lands (Metaxas et al. 2007). At approximately 14:15 (air temperature around 18 °C), we observed a single lacertid lizard running and hiding under some garbage at the side of a stream (37.974649°N, 23.688932°E; 18 m a.s.l.). After a while, the lizard emerged from its hiding place and was photographed. We did not encounter any other individuals and thus we returned to the spot a week later (6 December 2020). This time we found eight lizards and captured one

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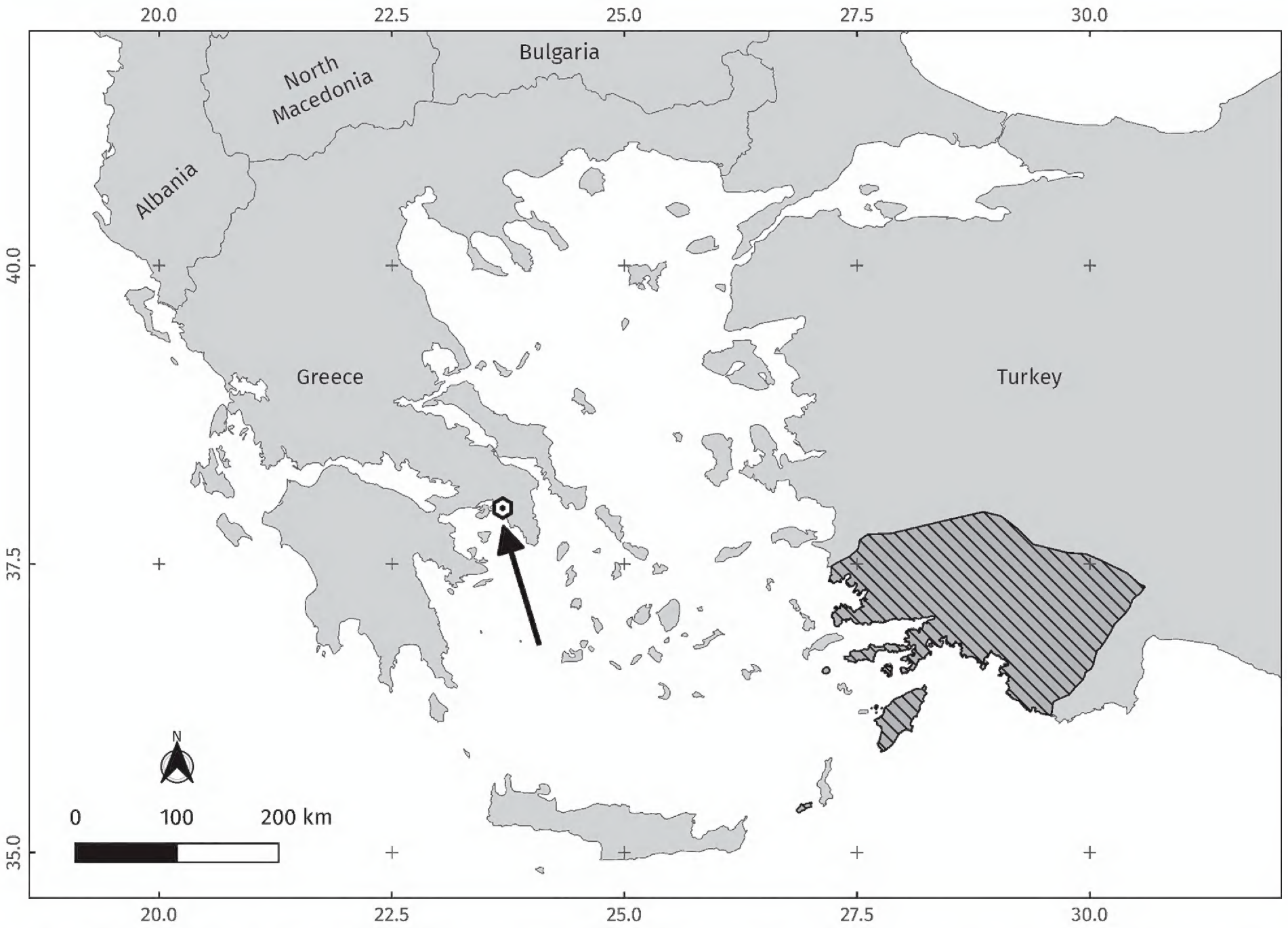

Figure 1. Known distribution of *Anatololacerta pelasgiana* (species range in gray). An arrow points to the new record in western Athens.

adult female (Fig. 2; SVL: 62 mm, tail length: 149 mm, body weight: 4.9 g). One month later (6 January 2021) we visited the spot again and counted 23 individuals (10 males, eight females, five juveniles) within a distance of about 300 meters along the stream. The presence of juveniles indicates that lizards do reproduce in this locality.

Based on the morphological characters and the coloration pattern of the captured individual and the ones we observed in the spot, we concluded that the lizards belonged to the genus *Anatololacerta*. To identify the species, we removed a tail tip (10 mm) from the captured female and sent it to the Molecular Systematics Lab of the Natural History Museum of Crete (NHMC) of the University of Crete. The captured specimen was deposited in the Herpetological Collection of the Zoological Museum of the National and Kapodistrian University of Athens (ZMUA 8624).

Total genomic DNA was extracted from the above specimen using a standard ammonium acetate protocol (Bruford et al. 1998). A fragment (~ 430 bp) of the mitochondrial gene (mtDNA) encoding for cytochrome *b* (cyt *b*), which is one of the most commonly amplified gene markers in the Lacertidae, was amplified through PCR using the primers GLUDG and CB2 (Palumbi 1996), following the conditions described in Karakasi et al. (2021). Double stranded sequencing was carried out using the Big-Dye Terminator v.3.1 Cycle Sequencing kit on an ABI3730 automated sequencer (CEMIA, Larissa, Greece), following the manufacturer's protocol and using the same primers as in PCR. Sequences were edited using CodonCode Aligner v.9.0.1 (CodonCode Corporation). The identity and authenticity of the produced sequence was evaluated with a BLAST search in the NCBI genetic database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). This search revealed that the produced cyt *b* sequence had high similarity with other available *cyt b* sequences of *Anatololacerta pelasgiana* in GenBank (E-value = 0).

To confirm the above results, we carried out a phylogenetic analysis using the data from Karakasi et al. (2021), which is the most recent and complete dataset including all species of *Anatololacerta* from the eastern Mediterranean region (for more details see Table 1). The alignment of the sequences was performed using the ClustalW implemented in MEGAX v.10.2.2 (Kumar et al. 2018), whereas the best model of nucleotide substitution was selected using the PartitionFinder (PF) v.2.1 (Guindon et al. 2010; Lanfear et al. 2012; Lanfear et al. 2016). The pairwise distances (p-distances) were estimated in MEGAX. Bayesian Inference (BI) was performed in MrBayes v.3.2.7 (Ronquist et al. 2012), with four runs and eight chains for each run. Each chain ran for 10^7 generations sampling every 10^3 generations. Several MCMC convergence diagnostics were used to check for convergence and stationarity following the manual's instructions. The

Figure 2. The captured individual, in situ photography (**A**) and in hand (**B**).

first 25% trees were discarded as burn-in, as a measure to sample from the stationary distribution and avoid the possibility of including random, sub-optimal trees. A majority rule consensus tree was then produced from the posterior distribution of trees, and the posterior probabilities were calculated as the percentage of samples recovering any particular clade. Posterior probabilities ≥0.95 indicate statistically significant support (Huelsenbeck and Ronquist 2001).

In total, 408 base pairs (bp) of cyt *b* sequence were obtained from the examined specimen collected in Athens (Accession number in GenBank: OP831897). Pairwise genetic distances (p-distance) between the specimen from Athens and all the others varied from 0 (from *A. pelasgiana* from the island of Rhodes) to 9.8% (from *A. ibrahimi* from Turkey), but were above 14.6% when compared with the outgroup taxa (*Phoenicolacerta*, *Iberolacerta*, *Lacerta*, *Parvilacerta*, and *Hellenolacerta*). The best-fit nucleotide substitution model selected by PF was HKY+I+G. In BI (harmonic mean -*lnL*= -4844.13), the MCMC convergence diagnostics did not provide any clues of non-convergence and indicated stationarity. Considering the *Anatololacerta* specimen is from Athens, it forms a highly supported clade [posterior probability (p.p.) = 1.00] with *A. pelasgiana* from Dodekanisa (Rhodes, Symi) and Turkey, showing closer proximity with the specimens of *A. pelasgiana* from Rhodes (Fig. 3).

The new Athenian home suits the Pelasgian lizard well. The stream along the banks of which we found the small population is a degraded torrent stream's bed bounded between two stone-built walls, approximately 2.5 meters high, comprising plenty of shelters and basking sites (Fig. 4). The stream is flowing most of the year, attracting insects and thus providing invertebrate food to the lizards. The vegetation is relatively sparse, with dominant species the Jerusalem thorn (*Parkinsonia aculeata*), black poplar (*Populus nigra*), castor bean (*Ricinus communis*), tree of heaven (*Ailanthus altissima*) and fig tree (*Ficus carica*). Though *A. pelasgiana* has been reported twice in the past to expand its range on other islands such as Kasos (Kornilios and Thanou 2016) and Kastelorizo (Kalaentzis et al. 2018), this is the first time it has settled in a mainland site within a large city.

Though we don't know the exact origin of the new population (based on the Bayesian Inference tree we hypothesize that it comes from Rhodes), we presume that the Pelasgian lizards arrived in Athens through one of the many transport and logistics companies that are located in the area, receiving and shipping goods to and from destinations all over the country. Reptilian unintentional human-mediated transportation is a well-known avenue of new introductions (Krysko and MacKenzie-Krysko 2016; Santos et al. 2018; Medina et al. 2019; Bisbal-Chinesta et al. 2020; Oskyrko et al. 2020), a case particularly common in large metropolitan centers (Tiatragul et al. 2020; Vaughn et al. 2021). Settling in Athens makes *A. pelasgiana* the eighth lizard species reported to have established population in the Greek capital city during the last seven years (*Podarcis siculus* – Adamopoulou 2015; *Chamaeleo chamaeleon* – Dimaki et al. 2015; *Podarcis muralis* – Karameta and Pafilis 2017; *Podarcis peloponnesiacus* – Hedman et al. 2017; *Podarcis vaucheri* – Spilani et al. 2018; *Tarentola mauritanica* – Strachinis and Pafilis 2018; *Algyroides nigropunctatus* – Deimezis-Tsikoutas et al. 2020). The adaptations in urban life that these new populations will adopt and their possible future interactions is a fascinating topic inviting further research (Sol et al. 2013; Johnson and Munshi-South 2017).

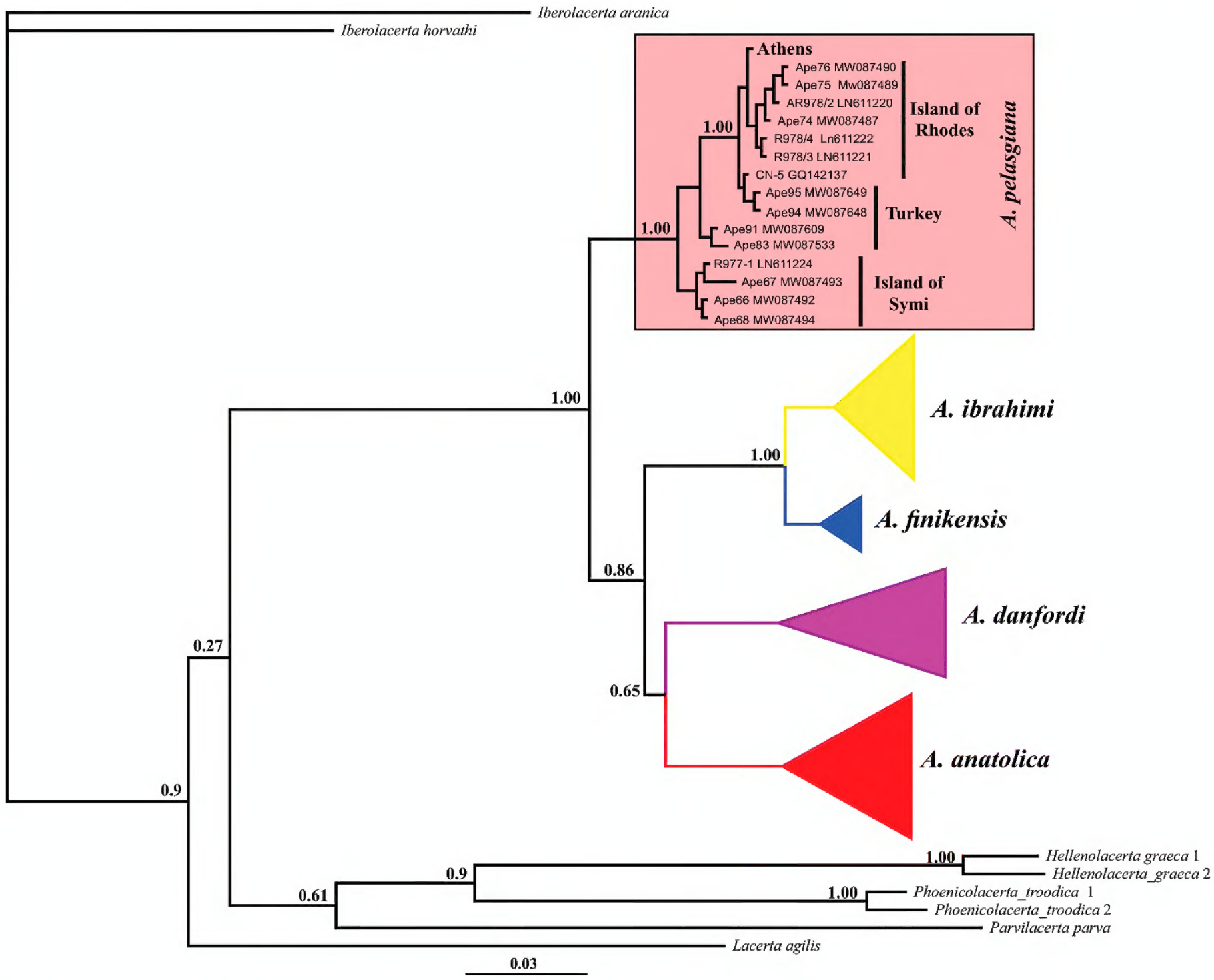

Figure 3. Bayesian Inference tree based on cyt *b* sequences. The posterior probabilities are given above the branches.

Figure 4. The habitat where the Pelasgian wall lizards were found in Profitis Daniil Stream.

Table 1. List of specimens examined in the present study with their corresponding taxon names, voucher numbers, country/region/locality names (detailed only where available), reference of the study in which they were previously used (if any), coordinates given in decimal degrees and accession numbers in GenBank. Clade assignment based on Karakasi et al. (2021).

Code	Clade	Species	Locality	Coordinates (Longidute, Latitude	cyt *b*	Reference
1	Clade B	*A. pelasgiana*	Athens, Greece	23.68893, 37.97464	OP831897	Present study
2	Clade A	*A. anatolica*	Turkey: Kaz Dag: Gure	26.88096, 39.62310	LN611203	Bellati et al. (2015)
3	Clade A	*A. anatolica*	Turkey: Kaz Dag: Gure	26.88096, 39.62310	LN611204	Bellati et al. (2015)
4	Clade A	*A. anatolica*	Turkey: Izmir: Karagol	27.21653, 38.55811	MW087597	Karakasi et al. (2021)
5	Clade A	*A. anatolica*	Turkey: Aydin: Pasayaylasi	27.88985, 37.93787	LN611207	Bellati et al. (2015)
6	Clade A	*A. anatolica*	Turkey: Aydin: Pasayaylasi	27.89219, 37.94394	MW087630	Karakasi et al. (2021)
7	Clade A	*A. anatolica*	Turkey: Aydin: Pasayaylasi	27.89219, 37.94394	MW087631	Karakasi et al. (2021)
8	Clade A	*A. anatolica*	Greece: Samos	26.89663, 37.73897	LN611212	Bellati et al. (2015)
9	Clade A	*A. anatolica*	Greece: Samos	26.66754, 37.78866	LN611210	Bellati et al. (2015)
10	Clade A	*A. anatolica*	Greece: Ikaria isl.	26.06290, 37.61707	LN611214	Bellati et al. (2015)
11	Clade A	*A. anatolica*	Greece: Ikaria isl.	26.05140, 37.56600	MW087496	Karakasi et al. (2021)
12	Clade B	*A. pelasgiana*	Greece: Symi isl.	27.84900, 36.60460	MW087492	Karakasi et al. (2021)
13	Clade B	*A. pelasgiana*	Greece: Symi isl.	27.84900, 36.60460	MW087493	Karakasi et al. (2021)
14	Clade B	*A. pelasgiana*	Greece: Symi isl.	27.84900, 36.60460	MW087494	Karakasi et al. (2021)
15	Clade B	*A. pelasgiana*	Greece: Symi isl.	27.83000, 36.62000	LN611224	Bellati et al. (2015)
16	Clade B	*A. pelasgiana*	Greece: Rodos isl.	27.94270, 36.28960	MW087487	Karakasi et al. (2021)
17	Clade B	*A. pelasgiana*	Greece: Rodos isl.	28.22115, 36.44388	MW087489	Karakasi et al. (2021)
18	Clade B	*A. pelasgiana*	Greece: Rodos isl.	28.22115, 36.44388	MW087490	Karakasi et al. (2021)
19	Clade B	*A. pelasgiana*	Greece: Rodos isl.	28.21767, 36.43549	GQ142137	Pavlicev and Mayer (2009)
20	Clade B	*A. pelasgiana*	Greece: Rodos isl.	28.21000, 36.33000	LN611220	Bellati et al. (2015)
21	Clade B	*A. pelasgiana*	Greece: Rodos isl.	28.21000, 36.33000	LN611221	Bellati et al. (2015)
22	Clade B	*A. pelasgiana*	Greece: Rodos isl.	28.21000, 36.33000	LN611222	Bellati et al. (2015)
23	Clade B	*A. pelasgiana*	Turkey: Antalya: Elmali	29.80464, 36.52431	MW087533	Karakasi et al. (2021)
24	Clade B	*A. pelasgiana*	Turkey: Antalya: Korkuteli	30.02850, 37.13719	MW087609	Karakasi et al. (2021)
25	Clade B	*A. pelasgiana*	Turkey: Denizli: Tavas	29.11686, 37.59461	MW087648	Karakasi et al. (2021)
26	Clade B	*A. pelasgiana*	Turkey: Denizli: Tavas	29.11686, 37.59461	MW087649	Karakasi et al. (2021)
27	Clade C	*A. finikensis*	Greece: Kastellorizo: Psomi	29.63720, 36.11530	MW087500	Karakasi et al. (2021)
28	Clade C	*A. finikensis*	Turkey: Antalya: near Sarilar	29.76800, 36.22376	LN611230	Bellati et al. (2015)
29	Clade C	*A. finikensis*	Turkey: Antalya: Altinyaka	30.40508, 36.68172	MW087646	Karakasi et al. (2021)
30	Clade C	*A. finikensis*	Turkey: Antalya: Karaman	30.16038, 36.94415	LN611228	Bellati et al. (2015)
31	Clade D	*A. ibrahimi*	Turkey: Burdur: Yakakoy	30.34522, 37.69956	MW087524	Karakasi et al. (2021)
32	Clade D	*A. ibrahimi*	Turkey: Antalya: Manavgat	31.54650, 36.88653	MW087586	Karakasi et al. (2021)
33	Clade D	*A. ibrahimi*	Turkey: Antalya: Manavgat	31.55906, 36.86689	MW087624	Karakasi et al. (2021)
34	Clade D	*A. ibrahimi*	Turkey: Antalya: Gazipasa	32.45828, 36.49711	MW087572	Karakasi et al. (2021)
35	Clade D	*A. ibrahimi*	Turkey: Mersin: Anamur	32.83011, 36.21247	MW087481	Karakasi et al. (2021)
36	Clade D	*A. ibrahimi*	Turkey: Icel: Abanoz	32.95107, 36.32493	LN611237	Bellati et al. (2015)
37	Clade D	*A. ibrahimi*	Turkey: Mersin: Gulnar	33.37964, 36.27850	MW087583	Karakasi et al. (2021)
38	Clade D	*A. ibrahimi*	Turkey: Mersin: Gulnar	33.37964, 36.27850	MW087585	Karakasi et al. (2021)
39	Clade E	*A. danfordi*	Turkey: Icel: Kavaklipinar	34.35123, 37.01912	LN611241	Bellati et al. (2015)
40	Clade E	*A. danfordi*	Turkey: Mersin: Camliyayla	34.57811, 37.14658	MW087638	Karakasi et al. (2021)
41	Clade E	*A. danfordi*	Turkey: Mersin: Camliyayla	34.57811, 37.14658	MW087640	Karakasi et al. (2021)
42	Clade E	*A. danfordi*	Turkey: Adana: Kozan	35.86014, 37.50894	MW087613	Karakasi et al. (2021)
43	Clade E	*A. danfordi*	Turkey: Adana: Feke	35.95111, 37.82644	MW087552	Karakasi et al. (2021)
44	Clade E	*A. danfordi*	Turkey: Adana: Feke	35.95111, 37.82644	MW087555	Karakasi et al. (2021)
45	Clade E	*A. danfordi*	Turkey: Adana: Saimbeyli	36.09392, 38.00275	MW087636	Karakasi et al. (2021)
46	Outgroup	*Iberolacerta aranica*	France: Serre de Ventaillou	–	AY151876	Carranza et al. (2004)
47	Outgroup	*Iberolacerta horvathi*	Croatia: Northwest	–	AY256648	Carranza et al. (2004)
48	Outgroup	*Hellenolacerta graeca* 1	Greece: Mystras	22.37201, 37.07046	LN611249	Bellati et al. (2014)
49	Outgroup	*Hellenolacerta graeca* 2	Greece: Serveika	22.31710, 37.15508	LN611250	Bellati et al. (2014)
50	Outgroup	*Phoenicolacerta troodica* 1	Cyprus: Kakopetria	32.91028, 34.97667	MW087633	Karakasi et al. (2021)
51	Outgroup	*Phoenicolacerta troodica* 2	Cyprus: Kakopetria	32.91028, 34.97667	MW087634	Karakasi et al. (2021)
52	Outgroup	*Lacerta agilis*	Greece: Makedonia: Florina	–	KJ940308	Sagonas et al. (2014)
53	Outgroup	*Parvilacerta parva*	Turkey: Sivas: Osmandede	37.02020, 38.74674	LN611248	Bellati et al. (2014)

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